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**14. ABSTRACT**

I.m. injection of HuBuChE is the only applicable method for enzyme administration under operational conditions. Pharmacokinetic studies in guinea pigs revealed that the Cmax of the enzyme was reached at 24 h after administration of the enzyme. At that time approx. 30% of the amount of administered enzyme was present in blood. It is still unknown whether the remainder of the enzyme is available in the tissues, which may explain the protective ratios found in earlier studies. In this bioavailability study the distribution of HuBuChE was examined after i.v. injection (100 % bioavailability) and i.m. administration. The enzyme was inhibited with $^{14}$C-soman, which facilitated the study of the distribution of only exogenous HuBuChE. At 24 h after i.v. administration of HuBuChE, 43 % of the administered amount was present in blood, while 33% of the administered amount was present in blood after i.m. administration. The increase of the HuBuChE concentration in richly perfused organs was rather low and accounted for less than 1 % for each organ to the total recovery. The recovery in intestines was rather high (5-8%), which might indicate that the exogenous enzyme was excreted in the feces. For pegylated HuBuChE, the recovery in blood at 24 h was 36 % after i.v. administration and only 17 % after i.m. administration. The increase of the concentration of pegylated HuBuChE in the tissues was also rather low. The contribution of each organ to the total recovery was less than 1%. The recovery of radioactivity in the intestines ranged between 2-6% of the administered amount, which directs to excretion of the enzyme.

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# Table of Contents

# I Introduction

Human serum butyrylcholinesterase (HuBuChE) is considered as a viable drug candidate for further development as a prophylactic antidote against organophosphate (OP)-based nerve agent toxicity. Pretreatment with HuBuChE alone has been demonstrated to confer full protection against OP intoxication [1-4]. HuBuChE is a stoichiometric scavenger that binds rapidly to nerve agents and moreover shows preference for the most toxic P(-)-isomers.

Recently, a mathematical toxicokinetic model was developed for the estimation of the dose of HuBuChE that is required to protect humans against the lethality of OPs [5]. Such a model requires basic knowledge of the volume of distribution of HuBuChE and a correlation between the distribution of HuBuChE in organs and tissues of experimental animals, with the levels of protection against OP intoxication. Under operational conditions, the enzyme is preferably administered intramuscularly because intravenous injection is not feasible. In the past, several pharmacokinetic studies in different species have been performed to show the build-up and elimination of BuChE in blood. The Cmax was reached at 10h or 24h after administration, depending on the species. Interestingly the amount of BuChE in blood of rodents and monkeys, calculated by multiplication of the volume of blood and the concentration of BuChE, was only approx. 16-30% of the total amount that was administered [4,6-8]. This value corresponds with a distribution volume of 20 -37% of body weight. Data from studies in humans indicate that the volume of distribution of HuBuChE at steady state ranges between 14-18% of body weight compared to a plasma volume of 5% [9, 10].

These findings are consistent with the remarkable ability of the tetrameric form of HuBuChE (approximately 340 kDa) to cross capillary walls following i.m. injections in rodents and monkeys [3,11], and suggest that the fast elimination phase of BuChE from blood of humans and monkeys following i.v. injections reflects distribution equilibrium in a relatively large volume of extra-vascular spaces. These results suggest that the volume of distribution of exogenously administered HuBuChE in humans is significantly greater than the volume of plasma. Thus, it is likely that HuBuChE levels in blood reflect only a part of the exogenously administered HuBuChE that is available for protecting against the adverse effects of OPs.

This finding was further verified in experiments where HuBuChE pretreated animals were exposed to OP nerve agents. HuBuChE pretreated guinea pigs were exposed to $2LCt_{50}$ soman vapor and remained signfree. Remarkably, the BuChE:soman ratio in blood was only 0.11, indicating that the distribution of HuBuChE outside the circulation is responsible for the sequestration of soman [4]. It is not clear to what extent the observed results are specific to the inhalation toxicokinetics of soman. However, protective ratios conferred by HuBuChE against an i.v. challenge of sarin, soman, tabun, and VX in mice were also found higher than the calculated values that were based on the scavenger level in blood. It was suggested that the small amount of extra-vascular HuBuChE, referred to as a non-vascular depot, could account for the observed differences [6].

Additionally, upon exposure to OP, HuBuChE will circulate as a HuBuChE-OP conjugate. The HuBuChE-OP conjugate has two fates: it can undergo spontaneous reactivation from nucleophilic attack by water or undergoes aging by loss of an R group from the bound OP. Since spontaneous reactivation is a very slow reaction, aging of the HuBuChE-OP conjugate is the more likely reaction. Due to the prolonged circulation of HuBuChE, HuBuChE-OP is also expected to circulate for long periods of time. Eventually, the aged HuBuChE-OP conjugate will be cleared from the circulation, most likely through the same clearance mechanisms as for HuBuChE. Although several studies have demonstrated the safety of HuBuChE alone, none of the studies so far have assessed the safety/toxicity of this aged HuBuChE-OP. It has to be studied whether the latter conjugate is safe.

Taken together, the possible role of extra-vascular HuBuChE and the fate of HuBuChE-OP complex merit a quantitative characterization.

A bioavailability study in which the distribution of exogenous administered HuBuChE-OP complex is studied by measuring the concentration of BuChE in tissues and blood after i.v. and i.m. administration should provide more insight into this question. This will not only permit the determination of mass balance of the injected enzyme, but also allow improvement of models aimed at predicting the dose of the enzyme required to protect against both bolus and long-term exposure to OPs.
Intramuscular injection of HuBuChE is the only applicable method for enzyme administration under operational conditions. Pharmacokinetic studies in guinea pigs revealed that the Cmax of the enzyme is reached at 24 h after administration of the enzyme. At that time approx. 30% of the amount of administered enzyme is present in blood. It is still unknown whether the remainder of the enzyme is available in the tissues, which may reveal a possible explanation for the protective ratios found in earlier studies. These protective ratios could not be explained by the levels of BuChE in blood alone. In this bioavailability study the distribution of HuBuChE was examined after i.v. injection (100 % bioavailability) and i.m. administration. Additionally, the toxicity/safety of the OP-inhibited enzyme is still unknown. In order to get a better insight into this question, the distribution between blood and tissues of the OP inhibited enzyme was investigated. For that purpose the enzyme was inhibited with $^{14}$C-soman, which facilitates to study the distribution of exogenously administered HuBuChE. The distribution of pegylated HuBuChE was also investigated in this study.

# II. Experimental

## II.1 Materials

$^{14}$C-soman (53 mCi/mmol) was obtained from the stocks of TNO Defense Security and Safety. (Pegylated) human butyrylcholinesterase was obtained from Dr. Saxena of Walter Reed Army Institute of Research (Silver Spring, MD, USA). Soluene 350 and Hionic Fluor were purchased from Perkin Elmer (Groningen, The Netherlands). 10 kD cut off filters (4 ml) were purchased from Millipore (Amsterdam, Netherlands). Radioactivity was quantified in a Tricarb 2900TR liquid scintillation counter (LSC) from Packard (Groningen, The Netherlands). Disodium hydrogen phosphate and potassium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Butyrylthiocholine iodide and 5,5'-dithiobis(2-nitrobenzoic acid) were purchased from Aldrich (Brussels, Belgium).

## II.2 Animals

Male albino outbred guinea pigs of the Dunkin-Hartley type (species identification: Crl:(HA)BR), weighing 350-400 g were purchased from Harlan NL (Horst, The Netherlands). Health certificates were examined before delivery was approved and were subsequently archived. The animals were housed in temperature- and humidity-controlled rooms. They were allowed to eat and drink *ad libitum*. Teklad® guinea pig food was procured from Harlan NL. Analysis reports of the food batches were received, inspected and filed. Two standard operation procedures were applicable to the care of the guinea pigs, i.e., 'Ordering and Housing of Experimental Animals' (SOP Q213-W-039) and 'Cleaning and Maintenance of Animal Facilities' (SOP Q213-W-040). The animals were allowed to acclimatize to their new environment for at least 1 week before they were used in any experiment. The protocol for the animal experiments was approved in September 2008 by the TNO Animal Experiment Committee under number DEC 2400.

## II.3 Preparation of $^{14}$C-soman inhibited BuChE

Human Butyrylcholinesterase (25 mg) was dissolved in 2.5 ml water and 2 equivalents of $^{14}$C-soman were added. After 30 min of incubation the inhibition of the enzyme activity was checked with the Ellman assay, using 0.4 mM butyrylthiocholine iodide as a substrate and 0.4 mM 5,5'-dithiobis(2-nitrobenzoic acid) in 0.05 M phosphate buffer, pH 8.0 at 25 °C.
The enzyme was divided over two 10 kD cut off filters and the enzyme was washed with phosphate buffered saline (4 ml each cycle) in order to remove the excess of $^{14}$C-soman. The washing procedure was repeated until the radioactivity in the wash fluid was less than 1 % of the radioactivity in the residue. The enzyme was recovered from the filter and the final concentration was adjusted to 10 mg/ml. $^{14}$C-soman labeled pegylated HuBuChE was prepared in the same way.

## II.4 Animal experiment

After an acclimatization period of one week in the housing facility, the animals were anesthetized with isoflurane. A cannula was installed in the vena jugularis. The labeled enzyme was i.v. injected via the cannula. The dose of $^{14}$C-soman labeled HuBuChE was 3 mg/kg corresponding with $4.5*10^6$ cpm/kg. The injection volume was 1 ml/kg. Animals that received HuBuChE i.m. were also anesthetized. The enzyme was injected i.m. in the hind leg. The dose was the same as for the animals that received the enzyme i.v.,

however the injection volume was 300 µl/kg. After the injection of the enzyme the animals were put back in their cage. After 24 h the animals were euthanized with an overdose of Nembutal.

## *II.5 Sample preparation*

Blood and the organs: brain, lung, heart, liver, spleen, kidney, eye and intestine were collected. A weighed aliquot of the organs (approx. 300 mg) was solubulized in 2 ml soluene for 48 h at 40 °C. When the tissue was completely dissolved a volume of 20 ml Scintillation fluid was added and the radioactivity was determined in the LSC. A volume of 50 µl blood was dissolved in scintillation fluid without any pre-preparation. Additionally 25% homogenates of the tissues in phosphate buffered saline were prepared. The homogenates were centrifuged and the fluid (500 µl) was applied on a Microcon 10kD cut off filter. After centrifugation for 30 min, 50% of the fluid had passed the filter. Volumes of 100 µl of filtrate and residue were mixed with scintillation fluid counted in the LSC. This procedure was performed to verify that the radioactive label was still conjugated to the enzyme.

# III Results and Discussion

## *III.1 Distribution of $^{14}$C-soman inhibited HuBuChE*

Animals were i.v. and i.m. injected with $^{14}$C-soman labeled HuBuChE. After 24 h blood and tissues were taken and the level of radioactivity was determined. Table 1 and 2 show the results for the i.v. administration and i.m. administration respectively. The total radioactivity in each organ was calculated by multiplying the measured radioactivity in dpm/mg and the weight of the tissue. It was assumed that blood contributes 6% to the body weight. Results for the individual animals can be found in Appendix 1.

**Table 1** Gross radioactivity in blood and organs of guinea pigs (n=5) after i.v injection with $^{14}$C-soman inhibited HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 36.399 | 1.256 | 713951 | 24001 | 43.1 | 2.25 |
| Brain | 0.784 | 0.026 | 2721 | 89 | 0.164 | 0.0062 |
| Lung | 9.093 | 0.517 | 17620 | 745 | 1.06 | 0.0519 |
| Heart | 7.648 | 0.525 | 8296 | 661 | 0.500 | 0.0418 |
| Liver | 5.751 | 0.429 | 71028 | 3681 | 4.27 | 0.208 |
| Spleen | 6.848 | 0.296 | 3089 | 187 | 0.185 | 0.0085 |
| Kidney | 9.656 | 0.474 | 29019 | 1467 | 1.74 | 0.0709 |
| Eyes | 0.926 | 0.069 | 635 | 52 | 0.038 | 0.0032 |
| Intestines | 4.356 | 0.580 | 222907 | 30318 | 13.3 | 1.71 |
| Total | | | 1069267 | 61203 | 64.5 | 4.4 |

**Table 2** Gross radioactivity in blood and organs of guinea pigs (n=6) after i.m injection with $^{14}$C-soman inhibited HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 27.618 | 1.763 | 556874 | 33257 | 33.3 | 1.9564 |
| Brain | 0.648 | 0.051 | 2257 | 155 | 0.135 | 0.0092 |
| Lung | 6.795 | 0.705 | 14474 | 1132 | 0.866 | 0.0681 |
| Heart | 6.476 | 0.459 | 7575 | 378 | 0.453 | 0.0231 |
| Liver | 4.046 | 0.302 | 56732 | 4101 | 3.39 | 0.2476 |
| Spleen | 5.382 | 0.946 | 2636 | 441 | 0.157 | 0.0265 |
| Kidney | 8.777 | 0.468 | 28427 | 1205 | 1.70 | 0.0721 |
| Eyes | 0.597 | 0.061 | 424 | 51 | 0.0254 | 0.0031 |
| Intestines | 2.986 | 0.279 | 161644 | 16556 | 9.66 | 0.9758 |
| Total | | | 831043 | 57277 | 49.7 | 3.38 |

Additionally, homogenates of the tissues were prepared. The supernatant was centrifuged using 10 kD filters as described in section II.5 to assure that $^{14}$C-label label was still conjugated to the enzyme. Figure 1 shows the results. It can be concluded that the main part of the radioactive label is still conjugated to the enzyme. Only in the kidney and lung a significant amount of radioactivity was recovered in the filtrate. It is possible that a low molecular radioactive impurity in the enzyme solution was accumulated in liver and kidney. Another possibility is that the enzyme was digested and that low molecular radioactive compounds were secreted via the kidney.

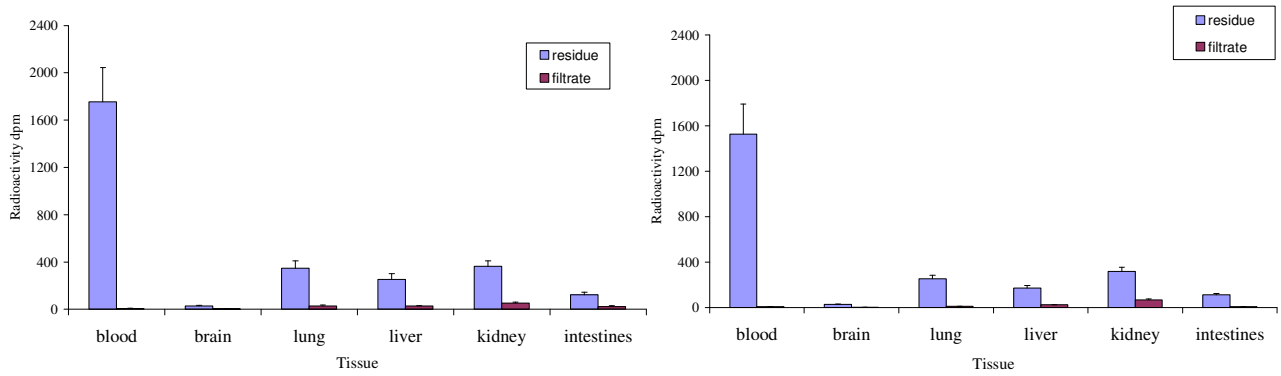


**Figure 1** Radioactivity in filtrate and residue of tissue homogenates after injection with $^{14}$C-soman inhibited HuBuChE . Left: i.v. injection, Right: i.m. injection

At 24 h after the i.v. injection approximately 43% of the injected amount was still present in the blood circulation. A limited amount is distributed into the tissues. The increase of radioactivity in the brain was almost negligible. Relative large organs and richly perfused organs such as liver, lung, and heart are responsible for 7% of the recovery of the administrated amount. It is remarkable, that the recovery in the intestines is almost 13% of the administrated amount.
It must be mentioned that the organs were not perfused with saline before they were removed. Since the radioactivity in blood is rather high the radioactivity in the organs is likely to be influenced by the blood content in that particular organ. For two reasons it was decided not to perfuse the organs. Firstly, the purpose of this experiment is to evaluate the difference between i.m. and i.v. injection. In that way the sample handling must be minimized to obtain a pure comparison. Secondly, the content of blood in organs of guinea pig tissue is known from literature. Using these values (see Table 3) the contribution of blood in that organ can be calculated and subtracted form the total radioactivity in that organ.

**Table 3** Blood content of guinea pig organs

| Tissue | weight % blood in tissue |
|---|---|
| Brain | 2.5 |
| Lung | 20 |
| Heart | 10 |
| Liver | 15 |
| Spleen | 11 |
| Kidney | 20 |
| Eyes | 2 |
| Intestines | 4 |

Data from Bosse and Wasserman (1970)[12]

Following this calculation Table 4 and 5 show the net radioactivity in blood and tissues.
Especially the liver and lung seems to be affected by this intervention. It is shown that the build-up of enzyme in the tissues is rather low and each organ contributes less than 1% to the total recovery. The total recovery of the enzyme is decreased from 64.5 to 52.9 %. The intestines are again an exception with a contribution of 8.6 % to the total recovery. It must be noted that the weight of the intestines was determined including the feces. The radioactivity in the intestines was determined in a part of the intestines without feces. So the weight of the feces, which could be estimated as 50% of the total weight of the intestines, will overestimate the amount of radioactivity in the intestines. Nevertheless a significant part of the total administrated amount was found in the intestines.

**Table 4** Net radioactivity in blood and organs of guinea pigs (n=5) after i.v. injection with $^{14}$C-soman inhibited HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 36.399 | 1.256 | 713951 | 24001 | 43.13 | 2.25 |
| Brain | -0.126 | 0.026 | -436 | 93 | -0.03 | 0.01 |
| Lung | 1.595 | 0.304 | 3067 | 547 | 0.19 | 0.03 |
| Heart | 4.008 | 0.450 | 4352 | 534 | 0.26 | 0.03 |
| Liver | 0.291 | 0.426 | 3241 | 4963 | 0.18 | 0.30 |
| Spleen | 2.625 | 0.228 | 1186 | 110 | 0.07 | 0.01 |
| Kidney | 2.449 | 0.535 | 7438 | 1637 | 0.44 | 0.09 |
| Eyes | 0.198 | 0.064 | 136 | 44 | 0.01 | 0.00 |
| Intestines | 2.827 | 0.610 | 144372 | 31114 | 8.63 | 1.76 |
| Total | | | 877306 | 63043 | 52.9 | 4.5 |

**Table 5** Net radioactivity in blood and organs of guinea pigs (n=6) after i.m injection with $^{14}$C-soman inhibited HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 27.618 | 1.763 | 556874 | 33257 | 33.3 | 1.96 |
| Brain | -0.043 | 0.022 | -153 | 78 | -0.01 | 0.00 |
| Lung | 1.106 | 0.531 | 2311 | 1028 | 0.14 | 0.06 |
| Heart | 3.714 | 0.360 | 4344 | 357 | 0.26 | 0.02 |
| Liver | -0.097 | 0.131 | -1266 | 1825 | -0.08 | 0.11 |
| Spleen | 2.179 | 0.831 | 1057 | 403 | 0.06 | 0.02 |
| Kidney | 3.309 | 0.257 | 10749 | 840 | 0.64 | 0.05 |
| Eyes | 0.045 | 0.039 | 34 | 29 | 0.00 | 0.00 |
| Intestines | 1.827 | 0.221 | 99116 | 12998 | 5.93 | 0.77 |
| Total | | | 673066 | 50815 | 40.2 | 3.0 |

## *III.2 Distribution of $^{14}$C-soman inhibited pegylated HuBuChE*

Analogous to the previous experiments, guinea pigs were injected i.v. and i.m. with $^{14}$C-soman inhibited pegylated HuBuChE. Table 6 and 7 show the results

**Table 6** Gross radioactivity in blood and organs of guinea pigs (n=6) after i.v. injection with $^{14}$C-soman inhibited pegylated HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM(%) |
|---|---|---|---|---|---|---|
| Blood | 30.28 | 0.78 | 607800 | 12210 | 36.47 | 0.73 |
| Brain | 0.76 | 0.05 | 2781 | 217 | 0.17 | 0.01 |
| Lung | 6.85 | 0.22 | 15114 | 931 | 0.91 | 0.06 |
| Heart | 5.73 | 0.35 | 6660 | 398 | 0.40 | 0.02 |
| Liver | 4.81 | 0.17 | 62808 | 3868 | 3.77 | 0.23 |
| Spleen | 6.04 | 0.33 | 3338 | 295 | 0.20 | 0.02 |
| Kidney | 8.99 | 0.54 | 28472 | 2336 | 1.71 | 0.14 |
| Eyes | 0.64 | 0.07 | 459 | 47 | 0.03 | 0.00 |
| Intestines | 3.47 | 0.31 | 163176 | 14801 | 9.79 | 0.89 |
| Total | | | 890608 | 35104 | 53 | 2 |

**Table 7** Gross radioactivity in blood and organs of guinea pigs (n=6) after i.m. injection with $^{14}$C-soman inhibited pegylated HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 14.93 | 1.06 | 294863 | 20460 | 17.69 | 1.23 |
| Brain | 0.37 | 0.04 | 1372 | 142 | 0.08 | 0.01 |
| Lung | 3.25 | 0.29 | 6664 | 630 | 0.40 | 0.04 |
| Heart | 2.48 | 0.29 | 2810 | 343 | 0.17 | 0.02 |
| Liver | 3.01 | 0.18 | 36515 | 2822 | 2.19 | 0.17 |
| Spleen | 2.65 | 0.33 | 1268 | 168 | 0.08 | 0.01 |
| Kidney | 6.37 | 0.24 | 18730 | 733 | 1.12 | 0.04 |
| Eyes | 0.26 | 0.03 | 184 | 18 | 0.01 | 0.00 |
| Intestines | 1.66 | 0.13 | 70823 | 4764 | 4.25 | 0.29 |
| Total | | | 433230 | 30081 | 20 | 2 |

Additionally, homogenates of the tissues were prepared and the supernatant was centrifuged using a Microcon 3kD cut off filter to verify that the $^{14}$C- label was still conjugated to the enzyme. Figure 2 shows the result. The main part of the $^{14}$C label is still conjugated to the enzyme, and the radioactivity in the filtrate of the kidney is again slightly elevated.

At 24 h the recovery of the enzyme in blood after i.v. injection was 36% which is lower than for HuBuChE (see Table 1). The recovery of pegylated enzyme in blood after i.m. injection was only 18% which is much lower than for HuBuChE, presumably because the migration of the pegylated enzyme from muscle tissue into blood proceeds less efficient than for the non-pegylated enzyme. The amount of radioactivity in the organs was again low, indicating that the distribution to these organs is reduced. Again the tissues were not perfused before they were removed. Table 8 en 9 show the net radioactivity in the organs, where the amount of radioactivity contributed by blood present in the organs is subtracted from the total radioactivity.

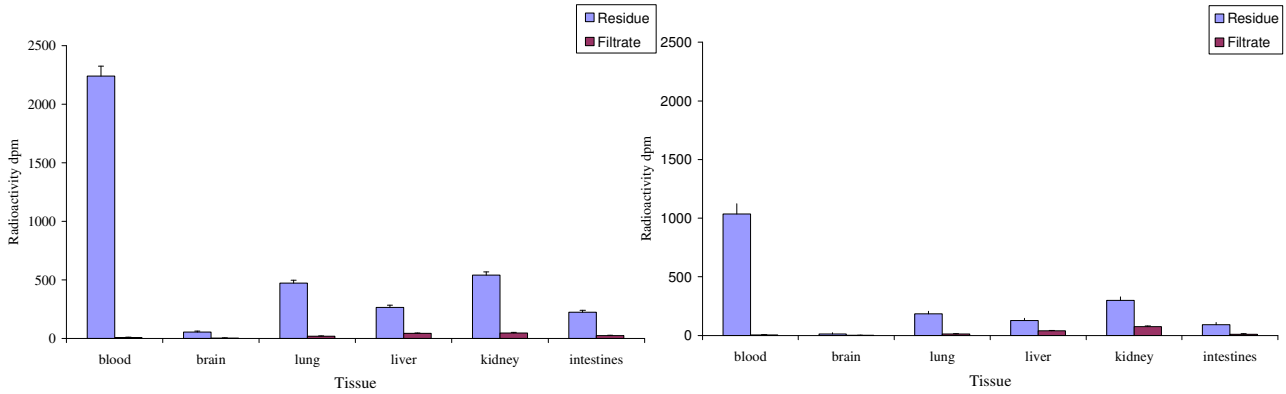


**Figure 2** Radioactivity in filtrate and residue of tissue homogenates after injection with $^{14}$C-soman inhibited HuBuChE. Left: i.v. injection, Right: i.m. injection

**Table 8** Net radioactivity in blood and organs of guinea pigs (n=6) after i.v. injection with $^{14}$C-soman inhibited pegylated HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 30.28 | 0.78 | 607800 | 12210 | 36.47 | 0.733 |
| Brain | 0.00 | 0.04 | 2761 | 217 | 0.00 | 0.009 |
| Lung | 0.61 | 0.34 | 13704 | 931 | 0.08 | 0.048 |
| Heart | 2.70 | 0.30 | 3521 | 398 | 0.19 | 0.021 |
| Liver | 0.27 | 0.07 | 59382 | 3868 | 0.21 | 0.055 |
| Spleen | 2.53 | 0.30 | 1949 | 295 | 0.08 | 0.011 |
| Kidney | 3.00 | 0.39 | 18935 | 2336 | 0.57 | 0.087 |
| Eyes | 0.04 | 0.06 | 434 | 47 | 0.00 | 0.003 |
| Intestines | 2.20 | 0.29 | 59666 | 14801 | 6.21 | 0.817 |
| Total | | | 730254 | 29716 | 44 | 2 |

**Table 9** Net radioactivity in blood and organs of guinea pigs (n=6) after i.m. injection with $^{14}$C-soman inhibited pegylated HuBuChE

| Organ | dpm/mg | SEM | dpm | SEM | Recovery (%) | SEM (%) |
|---|---|---|---|---|---|---|
| Blood | 14.93 | 1.0626 | 294863 | 20460 | 17.69 | 1.228 |
| Brain | 0.00 | 0.0288 | 1369 | 106 | 0.00 | 0.006 |
| Lung | 0.17 | 0.1013 | 6305 | 202 | 0.02 | 0.012 |
| Heart | 0.99 | 0.1934 | 1687 | 227 | 0.07 | 0.014 |
| Liver | 0.77 | 0.0778 | 27199 | 1003 | 0.56 | 0.060 |
| Spleen | 0.92 | 0.2191 | 828 | 107 | 0.03 | 0.006 |
| Kidney | 3.41 | 0.2258 | 8702 | 632 | 0.60 | 0.038 |
| Eyes | -0.04 | 0.0209 | 211 | 14 | 0.00 | 0.001 |
| Intestines | 1.03 | 0.0960 | 26779 | 3521 | 2.64 | 0.211 |
| Total | | | 360149 | 26272 | 22 | 2 |

The results in Table 8 and 9 show that the increase of the pegylated HuBuChE concentration in the organs is low. Only the radioactivity in the intestines contributes significant to the overall recovery. In these experiments the level of radioactivity was also determined in the feces. The average level of radioactivity in the feces was 2.5 ± 0.2 and 1.8 ± 0.2 dpm/mg for i.v. and i.m. administration, respectively. These levels

are higher than those found in the organs, which might indicate that the exogenous enzyme was excreted via the feces. In that respect it would be advisable to perform these experiments in metabolic cages to collect urine and feces and determine the radioactivity in these samples.
Concluding, at 24 h after injection of HuBuChE approximately 43 % is still present in blood after i.v. injections and 33% is present in blood after i.m. injection (see Fig. 3). The increase of the HuBuChE in the tissues is rather low and doesn't contribute significantly to the total recovery.
The recovery of pegylated HuBuChE in blood at 24 h after administration is significantly lower than after i.v. administration. The recovery in blood after i.v. administration is lower than obtained with HuBuChE, presumably because the elimination rate of HuBuChE is lower than for pegylated HuBuChE. However, for a better determination of the elimination rate it is advisable to perform kinetic studies. The total amount of radioactivity given to the animals (body weight approx. 320 g) was 1.6 x$10^6$ dpm. An equal distribution over the body would results in 5 dpm/mg. The level of radioactivity in all richly perfused organs was lower than 5 dpm/mg. It is unlikely that the rest of the body will contain a higher level than 5 dpm/mg. A careful conclusion is that the rest of the enzyme has already been excreted in urine and feces. Again this presumption should be confirmed in metabolic experiments, where feces and urine can be collected over 24h.

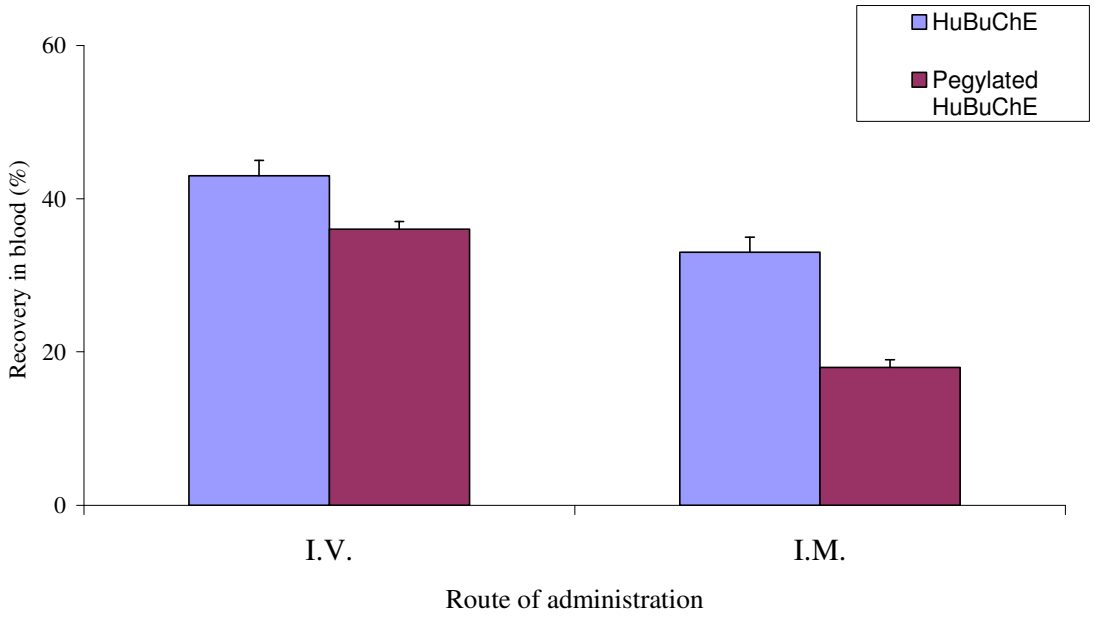


**Figure 3** Recovery of $^{14}$C-soman inhibited (pegylated) HuBuChE in blood of guinea pigs at 24 h after administration i.v. or i.m.

# IV Key Research Accomplishments

- (Pegylated) HuBuChE could be successful radio labeled by inhibition of the enzyme with $^{14}$C-soman. The excess of soman was removed by additional washing steps resulting in radio-purity enzyme of more than 99%. This enzyme was excellent useful to study the fate of exogenous HuBuChE.

- The recovery of HuBuChE in blood of guinea pig at 24 h after i.v. administration was 43 ± 2%.

- The recovery of HuBuChE in blood of guinea pig at 24 h after i.m. administration was 33 ± 2%.

- The recovery of pegylated HuBuChE in blood of guinea pig at 24 h after i.v. administration was 36 ± 2%.

- The recovery of pegylated HuBuChE in blood of guinea pig at 24 h after i.m. administration was 17 ± 1%, which is significantly lower than after i.v. administration. Possibly, the transport of the pegylated enzyme from the muscle tissue to blood proceeds less efficient.

- The increase of the (pegylated) HuBuChE concentration in the organs is very low after i.v. and i.m. administration (less than 1% for each tissue). Significant higher recoveries of radioactivity were found in the intestines, indicating that the enzyme might be excreted in the feces.

# V Reportable Outcomes

In cooperation with the Grants Officer Representative (GOR) dr. Saxena from WRAIR, it will be decided if and how this work can be published.

# VI Conclusion

Human serum butyrylcholinesterase (HuBuChE) is considered as a viable drug candidate for further development as a prophylactic antidote against nerve agent toxicity. Pretreatment with HuBuChE alone has been demonstrated to confer full protection against OP intoxication. HuBuChE is a stoichiometric scavenger that binds rapidly to nerve agents and, moreover, shows preference for the most toxic P(-)-isomers. Intramuscular injection of HuBuChE is the only applicable method for enzyme administration under operational conditions. Previously performed pharmacokinetic studies in guinea pigs i.m. injected with HuBuChE revealed that the Cmax of the enzyme was reached at 24 h after administration. At that time approx. 30% of the administered amount was present in the blood circulation. Since the fate of 70% of the administered amount is unknown, the distribution of exogenous administered (pegylated) HuBuChE was studied in this project. (Peglyated) HuBuChE was inhibited with $^{14}$C-soman to prepare radioactive labeled HuBuChE that could be followed as exogenous enzyme in the distribution studies. At 24 h after the i.v. administration of HuBuChE 43 % was present in blood, while 33% of the enzyme was present in blood after i.m. administration. The increase of the enzyme concentration in the tissues was rather low and the contribution to the total recovery was not more than 1 % for each tissue. Remarkably, the contribution of the intestines to the total recovery of radioactivity was almost 6%. Possibly, the exogenous enzyme was excreted. Additional metabolic studies, in which radioactivity in urine and feces can be examined should verify this finding. Similar conclusions were drawn after administration of pegylated HuBuChE, with the exception that the recovery in blood at 24 h after i.m. administration was much lower, 17%.

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# VIII Technical Objectives

T.O.-1 Preparation of $^{14}$C-soman inhibited BuChE
Plasma derived BuChE will be inhibited with $^{14}$C-soman. After inhibition is complete, excess $^{14}$C-soman will be removed by ultra filtration.

T.O.-2 Preparation of $^{14}$C-soman inhibited pegylated BuChE
Pegylated recombinant BuChE will be inhibited with $^{14}$C-soman. After inhibition is complete, excess $^{14}$C-soman will be removed by ultra filtration.

T.O.-3 Distribution of exogenous HuBuChE after intra-venous administration.
$^{14}$C-soman inhibited HuBuChE will be i.v. injected in guinea pigs (3 mg/kg). At 24 h after the administration, animals will be euthanized and the organs will be removed. The amount of radioactivity in blood, brain, lungs, heart, liver, spleen, kidney, eyes and intestines will be determined.

T.O.-4 Distribution of exogenous HuBuChE after intra-muscular administration
$^{14}$C-soman inhibited HuBuChE will be i.m. injected in guinea pigs (3 mg/kg). At 24 h after the administration, animals will be euthanized and the organs will be removed. The amount of radioactivity in blood, brain, lungs, heart, liver, spleen, kidney, eyes and intestines will be determined.

T.O.-5 Distribution of exogenous pegylated-HuBuChE after intra-venous administration
$^{14}$C-soman inhibited pegylated HuBuChE will be i.v. injected in guinea pigs (3 mg/kg). At 24 h after the administration, animals will be euthanized and the organs will be removed. The amount of radioactivity in blood, brain, lungs, heart, liver, spleen, kidney, eyes and intestines will be determined.

T.O.-6 Distribution of exogenous pegylated-HuBuChE after intra-muscular administration
$^{14}$C-soman inhibited pegylated HuBuChE will be i.m. injected in guinea pigs (3 mg/kg). At 24 h after the administration, animals will be euthanized and the organs will be removed. The amount of radioactivity in blood, brain, lungs, heart, liver, spleen, kidney, eyes and intestines will be determined.

# IX Appendix 1

## Distribution of $^{14}$C-soman inhibited HuBuChE

Animal 1 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 316 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 25 | 18.96 | 482262 | 28.9 |
| Brain | 0.67 | 3.47 | 2324 | 0.1 |
| Lung | 6.29 | 2.13 | 13408 | 0.8 |
| Heart | 6.77 | 1.15 | 7785 | 0.5 |
| Liver | 3.98 | 14.28 | 56808 | 3.4 |
| Spleen | 5.63 | 0.46 | 2588 | 0.2 |
| Kidney | 8.91 | 3.50 | 31168 | 1.9 |
| Eyes | 0.54 | 0.70 | 377 | 0.0 |
| Intestines | 3.23 | 52.67 | 169931 | 10.2 |
| Total | | | 766649 | 46.0 |

Animal 2 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 316 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 742 | 18.96 | 703175 | 42.2 |
| Brain | 401 | 3.35 | 2684 | 0.2 |
| Lung | 4326 | 1.97 | 17044 | 1.0 |
| Heart | 3711 | 0.93 | 6903 | 0.4 |
| Liver | 3738 | 11.33 | 84710 | 5.1 |
| Spleen | 2489 | 0.44 | 3221 | 0.2 |
| Kidney | 5814 | 2.88 | 33486 | 2.0 |
| Eyes | 509 | 0.72 | 719 | 0.0 |
| Intestines | 3026 | 40.54 | 245324 | 14.7 |
| Total | | | 1097267 | 65.8 |

Animal 3 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 321 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 32.33 | 19.26 | 622720 | 37.4 |
| Brain | 0.82 | 3.32 | 2730 | 0.2 |
| Lung | 10.17 | 1.94 | 19723 | 1.2 |
| Heart | 8.44 | 1.11 | 9365 | 0.6 |
| Liver | 5.24 | 14.41 | 75441 | 4.5 |
| Spleen | 9.80 | 0.49 | 4802 | 0.3 |
| Kidney | 10.69 | 3.02 | 32295 | 1.9 |
| Eyes | 0.87 | 0.76 | 663 | 0.0 |
| Intestines | 3.47 | 48.87 | 169755 | 10.2 |
| Total | | | 937494 | 56.2 |

Animal 4 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 309 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 39.05 | 18.54 | 723967 | 43.4 |
| Brain | 0.86 | 3.49 | 3003 | 0.2 |
| Lung | 10.84 | 1.88 | 20385 | 1.2 |
| Heart | 9.75 | 1.12 | 10919 | 0.7 |
| Liver | 5.99 | 11.99 | 71847 | 4.3 |
| Spleen | 7.61 | 0.44 | 3348 | 0.2 |
| Kidney | 9.27 | 2.86 | 26517 | 1.6 |
| Eyes | 1.04 | 0.74 | 773 | 0.0 |
| Intestines | 2.55 | 50.05 | 127702 | 7.7 |
| Total | | | 988462 | 59.3 |

Animal 5 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Weight | 324 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 647 | 19.44 | 628919 | 37.7 |
| Brain | 354 | 3.45 | 2442 | 0.1 |
| Lung | 3221 | 1.99 | 12820 | 0.8 |
| Heart | 3391 | 1.05 | 7121 | 0.4 |
| Liver | 2244 | 12.95 | 58121 | 3.5 |
| Spleen | 1813 | 0.40 | 2133 | 0.1 |
| Kidney | 4636 | 2.93 | 27168 | 1.6 |
| Eyes | 314 | 0.68 | 427 | 0.0 |
| Intestines | 1767 | 53.55 | 189236 | 11.4 |
| Total | | | 928389 | 55.7 |

Animal 6 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 320 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 35.54 | 19.20 | 682349 | 40.9 |
| Brain | 0.82 | 3.52 | 2877 | 0.2 |
| Lung | 8.83 | 2.05 | 18102 | 1.1 |
| Heart | 7.69 | 1.11 | 8533 | 0.5 |
| Liver | 5.30 | 13.51 | 71637 | 4.3 |
| Spleen | 7.09 | 0.39 | 2766 | 0.2 |
| Kidney | 9.58 | 3.02 | 28940 | 1.7 |
| Eyes | 0.63 | 0.69 | 436 | 0.0 |
| Intestines | 3.62 | 49.76 | 180335 | 10.8 |
| Total | | | 995975 | 59.8 |

Animal 7 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 352 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 21.51 | 21.12 | 454340 | 27.3 |
| Brain | 0.46 | 3.72 | 1694 | 0.1 |
| Lung | 5.15 | 2.31 | 11896 | 0.7 |
| Heart | 5.44 | 1.31 | 7133 | 0.4 |
| Liver | 3.09 | 16.31 | 50317 | 3.0 |
| Spleen | 3.60 | 0.57 | 2052 | 0.1 |
| Kidney | 7.42 | 3.31 | 24554 | 1.5 |
| Eyes | 0.48 | 0.73 | 351 | 0.0 |
| Intestines | 2.07 | 60.42 | 124991 | 7.5 |
| Total | | | 677329 | 40.6 |

Animal 8 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.75 *10$^6$ dpm | Body Weight | 345 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 31.52 | 20.70 | 652394 | 37.1 |
| Brain | 0.71 | 3.49 | 2465 | 0.1 |
| Lung | 7.44 | 2.07 | 15409 | 0.9 |
| Heart | 6.29 | 1.12 | 7047 | 0.4 |
| Liver | 5.13 | 13.03 | 66856 | 3.8 |
| Spleen | 5.85 | 0.62 | 3624 | 0.2 |
| Kidney | 9.22 | 3.42 | 31516 | 1.8 |
| Eyes | 0.93 | 0.67 | 624 | 0.0 |
| Intestines | 5.50 | 59.13 | 325464 | 18.5 |
| Total | | | 1105401 | 62.8 |

Animal 9 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.75 *10$^6$ dpm | Body Weight | 341 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 25.33 | 20.46 | 518178 | 31.1 |
| Brain | 0.57 | 3.36 | 1921 | 0.1 |
| Lung | 6.55 | 2.28 | 14924 | 0.9 |
| Heart | 5.86 | 1.22 | 7153 | 0.4 |
| Liver | 3.65 | 12.88 | 46951 | 2.8 |
| Spleen | 4.17 | 0.53 | 2210 | 0.1 |
| Kidney | 7.97 | 3.31 | 26378 | 1.6 |
| Eyes | 0.46 | 0.66 | 304 | 0.0 |
| Intestines | 2.16 | 47.89 | 103402 | 6.2 |
| Total | | | 721421 | 43.3 |

Animal 10 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.26 *10$^6$ dpm | Body Weight | 347 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 38.80 | 20.82 | 807869 | 52.0 |
| Brain | 0.74 | 3.50 | 2576 | 0.2 |
| Lung | 9.70 | 1.77 | 17162 | 1.1 |
| Heart | 7.09 | 1.14 | 8079 | 0.5 |
| Liver | 4.85 | 12.38 | 60090 | 3.9 |
| Spleen | 6.37 | 0.39 | 2484 | 0.2 |
| Kidney | 8.58 | 2.87 | 24634 | 1.6 |
| Eyes | 1.03 | 0.61 | 625 | 0.0 |
| Intestines | 4.05 | 58.23 | 235710 | 15.2 |
| Total | | | 1159230 | 74.7 |

Animal 11 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.37 *10$^6$ dpm | Body Weight | 368 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 28.75 | 22.08 | 634826 | 37.6 |
| Brain | 0.66 | 3.69 | 2432 | 0.1 |
| Lung | 6.17 | 2.28 | 14074 | 0.8 |
| Heart | 5.56 | 1.24 | 6891 | 0.4 |
| Liver | 3.84 | 13.73 | 52751 | 3.1 |
| Spleen | 3.76 | 0.54 | 2032 | 0.1 |
| Kidney | 8.40 | 3.45 | 28997 | 1.7 |
| Eyes | 0.60 | 0.70 | 422 | 0.0 |
| Intestines | 3.46 | 61.48 | 212549 | 12.6 |
| Total | | | 954974 | 56.5 |

Animal 12 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited HuBuChE

| Dose | 1.26 *10$^6$ dpm | Body Weight | 347 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 15.06 | 20.82 | 313577 | 20.2 |
| Brain | 0.35 | 3.83 | 1342 | 0.1 |
| Lung | 3.38 | 2.04 | 6896 | 0.4 |
| Heart | 2.77 | 1.26 | 3493 | 0.2 |
| Liver | 2.64 | 12.36 | 32629 | 2.1 |
| Spleen | 2.30 | 0.51 | 1174 | 0.1 |
| Kidney | 5.36 | 3.06 | 16394 | 1.1 |
| Eyes | 1.11 | 0.70 | 778 | 0.1 |
| Intestines | 1.84 | 52.73 | 97223 | 6.3 |
| Total | | | 473507 | 30.5 |

Results of animal 12 are not used in calculation of the mean values because the level in blood was exceptionally low.

## Distribution of $^{14}$C-soman inhibited pegylated HuBuChE

Animal 13 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 321 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 19.15 | 19.26 | 368776 | 22.1 |
| Brain | 0.44 | 3.57 | 1560 | 0.1 |
| Lung | 4.33 | 2.11 | 9136 | 0.5 |
| Heart | 3.44 | 1.07 | 3683 | 0.2 |
| Liver | 3.71 | 12.91 | 47842 | 2.9 |
| Spleen | 3.71 | 0.51 | 1890 | 0.1 |
| Kidney | 7.07 | 2.99 | 21144 | 1.3 |
| Eyes | 0.30 | 0.68 | 206 | 0.0 |
| Intestines | 2.17 | 41.09 | 89332 | 5.4 |
| Total | | | 543569 | 32.6 |

Animal 14 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 325 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 33.89 | 19.5 | 660822 | 39.6 |
| Brain | 0.87 | 3.66 | 3179 | 0.2 |
| Lung | 6.29 | 2.07 | 13021 | 0.8 |
| Heart | 7.00 | 1.14 | 7981 | 0.5 |
| Liver | 5.45 | 14.96 | 81582 | 4.9 |
| Spleen | 6.45 | 0.41 | 2644 | 0.2 |
| Kidney | 11.47 | 3.38 | 38773 | 2.3 |
| Eyes | 0.77 | 0.72 | 555 | 0.0 |
| Intestines | 4.22 | 43.09 | 181770 | 10.9 |
| Total | | | 990326 | 59.4 |

Animal 15 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 326 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 30.27 | 19.56 | 592037 | 35.5 |
| Brain | 0.93 | 3.83 | 3554 | 0.2 |
| Lung | 6.73 | 2.01 | 13527 | 0.8 |
| Heart | 5.88 | 1.13 | 6646 | 0.4 |
| Liver | 4.93 | 12.73 | 62747 | 3.8 |
| Spleen | 6.46 | 0.48 | 3103 | 0.2 |
| Kidney | 8.75 | 3.14 | 27461 | 1.6 |
| Eyes | 0.92 | 0.70 | 642 | 0.0 |
| Intestines | 4.43 | 47.22 | 209370 | 12.6 |
| Total | | | 919088 | 55.1 |

Animal 16 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 309 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 14.12 | 18.54 | 261811 | 15.7 |
| Brain | 0.41 | 3.73 | 1512 | 0.1 |
| Lung | 2.71 | 1.80 | 4871 | 0.3 |
| Heart | 2.37 | 1.04 | 2463 | 0.1 |
| Liver | 3.13 | 12.40 | 38781 | 2.3 |
| Spleen | 2.17 | 0.50 | 1083 | 0.1 |
| Kidney | 5.93 | 3.00 | 17790 | 1.1 |
| Eyes | 0.34 | 0.69 | 234 | 0.0 |
| Intestines | 1.78 | 40.09 | 71204 | 4.3 |
| Total | | | 399749 | 24.0 |

Animal 17 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 338 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 29.23 | 20.28 | 592796 | 35.6 |
| Brain | 0.68 | 3.43 | 2325 | 0.1 |
| Lung | 7.77 | 2.49 | 19354 | 1.2 |
| Heart | 5.05 | 1.18 | 5962 | 0.4 |
| Liver | 4.79 | 11.73 | 56187 | 3.4 |
| Spleen | 6.27 | 0.46 | 2884 | 0.2 |
| Kidney | 8.14 | 2.67 | 21737 | 1.3 |
| Eyes | 0.57 | 0.72 | 407 | 0.0 |
| Intestines | 2.38 | 42.22 | 100275 | 6.0 |
| Total | | | 801926 | 48.1 |

Animal 18 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 343 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 30.16 | 20.58 | 620702 | 37.2 |
| Brain | 0.66 | 3.73 | 2466 | 0.1 |
| Lung | 6.78 | 2.32 | 15736 | 0.9 |
| Heart | 6.14 | 1.22 | 7485 | 0.4 |
| Liver | 4.77 | 12.07 | 57563 | 3.5 |
| Spleen | 6.95 | 0.63 | 4378 | 0.3 |
| Kidney | 9.20 | 3.24 | 29820 | 1.8 |
| Eyes | 0.62 | 0.69 | 425 | 0.0 |
| Intestines | 3.37 | 48.75 | 164404 | 9.9 |
| Total | | | 902978 | 54.2 |

Animal 19 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 333 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 15.68 | 19.98 | 313247 | 18.8 |
| Brain | 0.35 | 3.67 | 1284 | 0.1 |
| Lung | 3.67 | 2.00 | 7330 | 0.4 |
| Heart | 3.01 | 1.24 | 3730 | 0.2 |
| Liver | 2.79 | 12.24 | 34124 | 2.0 |
| Spleen | 2.75 | 0.40 | 1101 | 0.1 |
| Kidney | 5.70 | 2.91 | 16578 | 1.0 |
| Eyes | 0.29 | 0.75 | 216 | 0.0 |
| Intestines | 1.53 | 42.73 | 65300 | 3.9 |
| Total | | | 442910 | 26.6 |

Animal 20 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 338 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 13.40 | 20.28 | 271751 | 16.3 |
| Brain | 0.27 | 3.67 | 1007 | 0.1 |
| Lung | 3.05 | 2.07 | 6306 | 0.4 |
| Heart | 2.29 | 1.20 | 2747 | 0.2 |
| Liver | 2.76 | 12.06 | 33335 | 2.0 |
| Spleen | 2.29 | 0.51 | 1169 | 0.1 |
| Kidney | 6.89 | 2.88 | 19837 | 1.2 |
| Eyes | 0.19 | 0.67 | 125 | 0.0 |
| Intestines | 1.60 | 44.98 | 72168 | 4.3 |
| Total | | | 408445 | 24.5 |

Animal 21 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 337 g | |
|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) |
| Blood | 29.89 | 20.22 | 604421 | 36.3 |
| Brain | 0.78 | 3.79 | 2946 | 0.2 |
| Lung | 6.44 | 2.24 | 14415 | 0.9 |
| Heart | 4.53 | 1.17 | 5302 | 0.3 |
| Liver | 4.73 | 12.37 | 58468 | 3.5 |
| Spleen | 4.84 | 0.60 | 2906 | 0.2 |
| Kidney | 8.75 | 3.14 | 27478 | 1.6 |
| Eyes | 0.54 | 0.66 | 357 | 0.0 |
| Intestines | 3.43 | 49.42 | 169659 | 10.2 |
| Total | | | 885952 | 53.2 |

Animal 22 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 333 g | | |
|---|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) | |
| Blood | 11.49 | 19.98 | 229477 | | 13.8 |
| Brain | 0.26 | 3.76 | 989 | | 0.1 |
| Lung | 2.34 | 2.26 | 5293 | | 0.3 |
| Heart | 1.36 | 1.08 | 1471 | | 0.1 |
| Liver | 2.46 | 11.04 | 27144 | | 1.6 |
| Spleen | 1.57 | 0.48 | 753 | | 0.0 |
| Kidney | 5.91 | 2.91 | 17197 | | 1.0 |
| Eyes | 0.18 | 0.73 | 135 | | 0.0 |
| Intestines | 1.18 | 45.43 | 53527 | | 3.2 |
| Total | | | 335985 | | 20.2 |

Animal 23 Radioactivity in blood and organs after i.v. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 324 g | | |
|---|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) | |
| Blood | 28.24 | 20.4 | 576025 | | 34.6 |
| Brain | 0.65 | 3.42 | 2212 | | 0.1 |
| Lung | 7.07 | 2.07 | 14630 | | 0.9 |
| Heart | 5.77 | 1.14 | 6583 | | 0.4 |
| Liver | 4.19 | 14.39 | 60300 | | 3.6 |
| Spleen | 5.28 | 0.78 | 4116 | | 0.2 |
| Kidney | 7.63 | 3.35 | 25562 | | 1.5 |
| Eyes | 0.45 | 0.82 | 369 | | 0.0 |
| Intestines | 2.99 | 51.44 | 153576 | | 9.2 |
| Total | | | 843374 | | 50.6 |

Animal 24 Radioactivity in blood and organs after i.m. administration of $^{14}$C-soman inhibited pegylated HuBuChE

| Dose | 1.66 *10$^6$ dpm | Body Weight | 343 g | | |
|---|---|---|---|---|---|
| Organ | dpm/mg | Weight (g) | dpm | Recovery (%) | |
| Blood | 15.75 | 20.58 | 324116 | | 19.4 |
| Brain | 0.51 | 3.66 | 1881 | | 0.1 |
| Lung | 3.39 | 2.08 | 7046 | | 0.4 |
| Heart | 2.41 | 1.15 | 2768 | | 0.2 |
| Liver | 3.22 | 11.77 | 37866 | | 2.3 |
| Spleen | 3.43 | 0.47 | 1611 | | 0.1 |
| Kidney | 6.72 | 2.95 | 19836 | | 1.2 |
| Eyes | 0.26 | 0.73 | 190 | | 0.0 |
| Intestines | 1.70 | 43.07 | 73404 | | 4.4 |
| Total | | | 468719 | | 28.1 |